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Low Antithrombin III Levels in Neonates with Idiopathic Respiratory Distress Syndrome: Poor Prognosis

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Summary

Automated microanalytic chromogenic coagulation assays allow serial monitoring of critically ill newborn infants. In this study 84 premature infants [26 healthy prematures and 58 neonates with idiopathic respiratory distress syndrome (IRDS)] were studied daily during the first week of life, to investigate the possible significance of hemostatic abnormalities in IRDS.

In neonates with IRDS, coagulation factors II and X, antithrombin III (AT-III), plasminogen, and α_2-antiplasmin were significantly lower than control values. Recovery of the initially low AT-III levels was delayed relative to the other coagulation parameters measured. An AT-III $\leq$ 0.15 U/ml was present within the first 6 h of life in eight patients who developed IRDS, seven of whom died within 48 h. Autopsy of these neonates showed widespread fibrin deposition and hemorrhage in vital organs consistent with intravascular coagulation. These findings indicate that very low levels of AT-III are associated with disseminated intravascular coagulation in neonates with IRDS and suggest that a deficiency of AT-III is predictive of a poor outcome.

Abbreviations

AT-III, antithrombin III
DIC, disseminated intravascular coagulation
IRDS, idiopathic respiratory distress syndrome
IVH, intraventricular hemorrhage

DIC is frequently encountered in premature newborn infants suffering from IRDS. Deficiencies in clotting factors and fibrinogen, increased levels of fibrinogen/fibrin degradation products and thrombocytopenia (2, 4, 19, 22, 23) have been reported to be caused by DIC. These coagulation abnormalities are associated with clinical symptoms of bleeding (7, 8, 16, 25, 29) particularly IVH and fibrin thrombi at autopsy (3, 7, 16, 31) suggesting that DIC contributes to the fatal outcome of this disease. Decreased levels of AT-III have been described in early stages of DIC in adult patients (6, 26). In cord blood samples of premature infants who developed IRDS, reduced AT-III levels have been reported (18, 21, 34); however, the relationship between AT-III activity and the clinical course of IRDS has not yet been thoroughly investigated. The availability of automated chromogenic coagulation assays enables the serial study of several coagulation parameters, and of AT-III in particular, in premature neonates with IRDS. These results are correlated with clinical signs and autopsy findings.

SUBJECTS AND METHODS

Subjects. The study group included 84 premature infants admitted to the neonatal intensive care unit of our hospital during the year 1981. Estimation of gestational age was based upon maternal dates and the Dubowitz scoring method (10). All neonates received vitamin K_1 (0.5–1.0 mg) (Konakion, Hoffman-La Roche, Basel, Switzerland) intramuscularly upon delivery. Newborn infants were subdivided into two groups (Table 1). Classification was made according to examination by a neonatologist (R. de Leeuw), who was unaware of the patient's coagulation profile. Control patients included 26 premature newborn infants who were in stable clinical condition. Birth weights were appropriate for gestational age. These infants served as a control group for those infants surviving IRDS. Birth weight and gestational age did not differ significantly (Table 1). Patients with IRDS (n = 58) fulfilled the following criteria: *1)* symptoms of respiratory distress (Silverman score > 3) within 1 h after birth and present for at least 24 h; *2)* reticulogranular pattern with air-bronchograms on chest x-ray; and *3)* evidence of right to left shunting determined by transcutaneous Po_2 determinaton and capillary blood gas analysis. Respiratory support included mechanical ventilation through endotracheal intubation followed by continuous positive air pressure (n = 33), or continuous positive air pressure only (n = 25). The duration of ventilatory support was greater than 7 d in 16 neonates, all of whom developed bronchopulmonary dysplasia. A standard postmortem examination of all organs was performed in infants who died within 48 h after birth. Histologic examinations were performed according to standard techniques including hematoxilin and eosin staining. Fibrin-related antigens were demonstrated using an indirect immunoperoxidase technique with affinity-purified antihuman fibrinogen IgG (11).

Sampling and methods. Blood samples were obtained from the umbilical or peripheral veins, or by heel puncture (24). The initial blood sample was obtained within 6 h after birth and subsequent samples were taken daily.

Blood (0.3 ml) was collected into polypropylene tubes (Greiner, Nürtingen, Germany) containing solid K_2EDTA (1.5 mg/ml). Plasma was prepared by centrifugation at 13,000 g for 4 min at room temperature. Automated chromogenic determinations of AT-III (20), factor X (29), factor II (24), plasminogen (24), and α_2-antiplasmin (24) could be performed with 70 μl of plasma. Normal adult and healthy term neonate values of these assays are 0.80–1.40 U/ml and 0.40–0.80 U/ml, respectively. Platelet counts were performed according to Feissly and Lüdin (12). Thrombocytopenia was defined as a platelet count below 100×10^9/l (13).

Table 1. *Means of birth wt and gestational age in the control group and both iodiopathic respiratory distress syndrome (IRDS) subgroups, ranges given in parentheses*

	Birth wt (g)	Gestational age (wk)
Control group (n = 26)	1740 (1220–2270)	31.9 (29–34)
IRDS group (n = 58)		
IRDS who died (n = 17)	1035 (635–1945)	28.6 (26–33)
IRDS who survived (n = 41)	1625* (810–2530)	30.8* (26–34)

* No statistically significant difference in comparison to the control group.

Statistical methods. Statistical evaluation of the data was performed using the one-way analysis of variance and Student's t test.

RESULTS

Coagulation investigations. The serial coagulation results in the control and IRDS groups who survived are shown in Figure 1. In the control group the mean plasma levels of factors II and X, AT-III, and plasminogen varied between 0.3 and 0.5 U/ml. In contrast, the α_2-antiplasmin concentration was close to the adult range. In all but one of the healthy prematures, the first day AT-III level was over 0.20 U/ml. The AT-III level of that particular infant normalized on the second day. During the first week, AT-III and factor II levels increased significantly. The mean plasminogen and α_2-antiplasmin levels did not change during the entire study period. Despite vitamin K_1 administration, factor X declined significantly to a minimum on d 6 ($P < 0.001$) for reasons not understood.

In both IRDS subgroups all initial coagulation parameters were significantly lower as compared with the control group ($P < 0.001$). The mean initial AT-III level of the neonates who died was 0.16 U/ml which was significantly lower as compared with the IRDS group which did survive (0.21 U/ml, $P < 0.01$). Seven of the eight neonates with IRDS and an initial AT-III level of less than 0.16 U/ml died. The mean plasma levels in the surviving IRDS group reached control values at different time intervals (Fig. 1). Factor X and α_2-antiplasmin reached control levels after 24 h whereas factor II and plasminogen were within the normal range on d 3 and 4, respectively. AT-III levels remained significantly lower until d 5. Fourteen neonates in the IRDS group (seven survivors, seven non-survivors) developed thrombocytopenia within the first 3 d and none of the control infants were thrombocytopenic.

Clinical signs of bleeding. A hemorrhagic tendency was present in 17 of 41 neonates surviving IRDS and in all neonates who subsequently died due to IRDS. Bleeding symptoms included skin hematomas, oozing from the umbilical cord or venipuncture sites, gastrointestinal bleeding, and IVH, diagnosed either clinically or by echography. No signs of bleeding were present in the control group.

Necropsy findings. Twelve neonates developing IRDS died within 48 h of birth and five died between the third and the sixteenth day. The necropsy findings in 10 newborns, who died within 48 h, and for whom autopsy permission was obtained, are summarized in Table 2. The clinical diagnosis of IRDS was confirmed by the presence of hyaline membranes (using routine staining procedures) in all cases.

In seven cases, fibrin deposition was observed within the hyaline membranes (Fig. 2). In eight neonates, fibrin thrombi were diffusely present in the microcirculation of the kidneys, liver, or lungs. The clinical diagnosis of IVH was confirmed in six neonates. In all neonates some form of cerebral hemorrhagic diathesis was present, including meningeal petechiae, subarachnoidal hemorrhage or IVH. In addition, hemorrhage in the lungs, kidneys and adrenal glands was present in four cases.

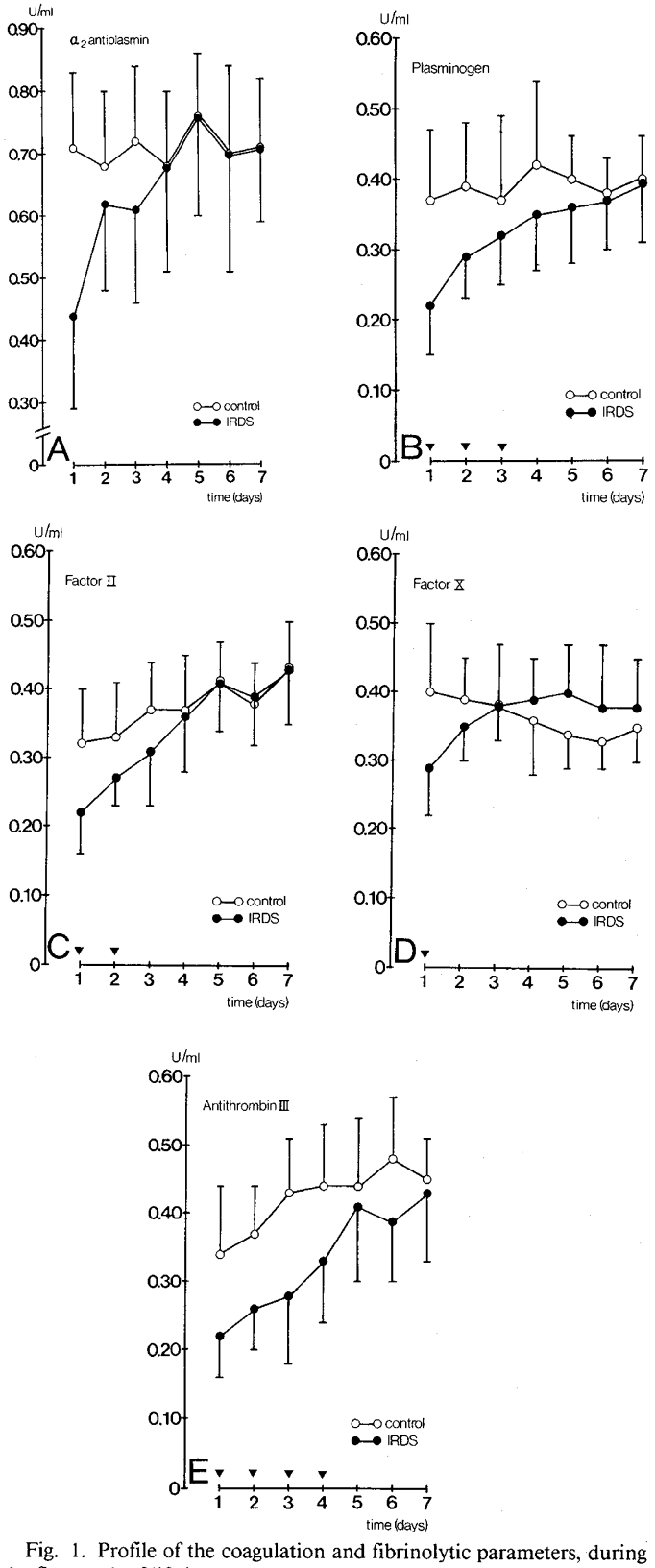

Fig. 1. Profile of the coagulation and fibrinolytic parameters, during the first week of life in healthy neonates (–O–), neonates with idiopathic respiratory distress syndrome (IRDS) who survived (–●–) (mean + SD for controls, mean − SD for IRDS). The days at which statistically significantly different values between healthy neonates and those with IRDS are noted have been marked with ▼.

Table 2. *Necropsy findings in neonates with IRDS who died within 48 h after birth*

No.	Birth wt (g)	Gestational age (wk)	AT-III U/ml d 1	AT-III U/ml d 2	Fibrin deposition and thrombi	Hemorrhage
1	1240	27	0.14*	...	liver	cerebral petechiae
2	1170	27	0.04*	...	hyaline membrane, liver	IVH, subarachnoid hemorrhage
3	1200	28	0.16*	...	liver	cerebral petechiae
4	1140	31	0.03*	...	hyaline membrane, liver	cerebral petechiae
5	1120	28	0.15*	...	hyaline membrane, liver	IVH
6	1200	30	0.12	0.24*	hyaline membrane, liver kidneys	cerebral petechiae, IVH, kidneys
7	1200	32	0.10	0.22*	lungs, kidneys	IVH, adrenal glands, kidneys
8	635	27	0.12*	...	hyaline membrane	cerebral petechiae, IVH, lungs
9	840	31	....	0.28*	hyaline membrane	cerebral petechiae, IVH, lungs
10	1010	32	0.19*	...	hyaline membrane	cerebral petechiae

* Deceased.

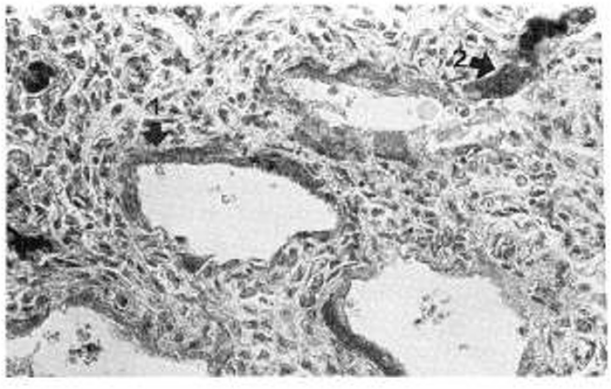

Fig. 2. Fibrin deposition in hyaline membranes (→ 1) and fibrin thrombi in the microcirculation (→ 2) of a premature infant who died due to idiopathic respiratory distress syndrome.

DISCUSSION

In this comprehensive study, severe coagulation abnormalities were demonstrated in premature infants suffering IRDS. Marked deficiencies of coagulation factors II and X and of plasminogen, as well as of the inhibitors of coagulation (AT-III) and fibrinolytic systems (α_2-antiplasmin) were observed and were associated with hemorrhage and fibrin thrombi in vital organs at autopsy.

IRDS was defined using a combination of clinical criteria, chest x-ray interpretation, and laboratory parameters in 58 premature newborn infants. Autopsy of 10 newborns who died within 48 h after birth revealed cerebral hemorrhage in all whereas diffuse hemorrhages in vital organs was noted in four cases. Fibrin thrombi, as visualized by specific antiserum staining, in lungs, liver, kidneys, or fibrin deposition in the hyaline membranes were observed in all cases. Considering these histologic findings, DIC obviously contributed to the fatal clinical course. It was, therefore, of particular interest to find very low AT-III levels in these infants as compared with the survivors. AT-III was the only parameter which distinguished between survivors and non-survivors in the IRDS group.

This severe AT-III deficiency, also observed in cord blood of such infants by other authors (18, 21, 34), may be the result of both increased turnover in the course of DIC and decreased synthesis due to liver damage. An acquired AT-III deficiency state may, in turn, accelerate the process of DIC and fibrin deposition in the microcirculation. The observed low plasminogen and α-antiplasmin levels may be suggestive of reactive fibrinolysis. This is in accordance with the findings of other authors (2, 4, 19, 22, 33). The coagulation changes and thrombocytopenia noted are compatible with the observed bleeding tendencies. The high incidence of IVH has also been asociated with coagulation changes (28) although other etiologies are also suggested, *i.e.,* inconsistency of blood pressure, loss of cerebral autoregulation, vascular immaturity, and changes in cardiac output (9, 31). Several therapeutic regimens have been investigated in an attempt to decrease mortality and to combat coagulation disorders in IRDS infants. Prophylactic heparin treatment was not successful (14, 23) and a definite heparin resistance has been observed (18, 23). The failure of heparin treatment in these infants might be ascribed to an AT-III deficiency, as the efficacy of heparin is mainly dependent on this inhibitor. Substitution of plasma alone has also been found to be ineffective (15) as was treatment with plasminogen concentrates (1). As AT-III is the most important physiologic inhibitor of blood coagulation and as the AT-III deficiencies are quite pronounced, it is anticipated that human AT-III concentrates, which were effective in previous clinical studies in adults (5, 27, 28), may be effective in the prevention or treatment of DIC associated with IRDS. We are currently involved in a double blind randomized study, comparing AT-III concentrates with placebo administration in these premature newborn infants.

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Glucose Turnover Rates in Chronically Catheterized Non-Pregnant and Pregnant Rabbits

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Summary

Glucose turnover rates have been measured in conscious, chronically catheterized, non-pregnant and pregnant rabbits. Non-pregnant rabbits were studied weekly for 4 wk. Pregnant animals were studied once while non-pregnant and then weekly for up to 4 wk during pregnancy. Glucose turnover rate was measured using a primed-constant infusion of [U-^{14}C]glucose and [6-^{3}H]glucose.

The weight of the rabbits did not vary throughout the 4–5 wk of study in either the non-pregnant or pregnant group. Seven pregnant rabbits delivered pups which weighed an average of 61 g each.

In non-pregnant rabbits, blood glucose concentration did not vary with time. In the pregnant rabbits, blood glucose concentration fell by the end of gestation to an average value of 74.6 ± 2.7 mg/dl, significantly less ($P < 0.01$) than the glucose concentration in the same animals before pregnancy, 88.2 ± 2.4 mg/dl.

The weight specific glucose turnover rate did not vary with time in either the non-pregnant (4.38 ± 0.16 mg·min^{-1}·kg^{-1}) or pregnant rabbits (3.89 ± 0.29 mg·min^{-1}·kg^{-1}). Blood glucose clearance did not change over time in the non-pregnant rabbits but did increase in the pregnant rabbits in late pregnancy. Blood glucose clearance was inversely related to the fall in blood glucose concentration.

Glucose turnover rates have been measured during late pregnancy in sheep, guinea pigs, rats, and women. In sheep, weight-specific glucose turnover rates for twin-pregnant ewes have been reported to average nearly 30% higher than for non-pregnant ewes (2). In pregnant guinea pigs studied over the last half of